STUDIES ON CITRUS BLACK SPOT

1. Infection of Fruit by the Causal Fungus

by

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INTRODUCTION

According to McOnie[1] in South Africa, the perithecia of black spot causal fungus, *Guignardia citricarpa* on dead leaves are the major source of inoculum for disease production while spores of the *Phoma* stage are relatively unimportant. In Taiwan, Chiu[2] reported that the causal fungus is present in apparently healthy fruits and leaves, and the latent fungus in fruit is observed as soon as flower petals fall. However, since no perithecia of the fungus on orchard floor had been recognized, he concluded that the infection of fruit is caused by pycnidiospores and the infection occurs at early stage of developing fruit.

This paper presents the preliminary results of studies on the infection of fruit by the causal fungus and also the results of general epidemiological investigations.

MATERIAIS AND METHODS

Investigations on the ascigerous stage of the citrus balck spot pathogen in the island were carried out in May, 1965. Dead citrus leaves were collected from the orchard floor at various locations, and examined for the presence of perithecia. Spraying experiments and bagging trials were conducted at Hsin-Pu, a Ponkan growing district, to determine the time of infection of fruit by the causal fungus. Difolatan W-80 at the concentration of 2 grams per liter was used in the staggered sprays. Ponkan trees were in full flower during the third week of March and 10 fruits on each of 20 unsprayed tress were covered with paper bags at several intervals. To ascertain the actual infection, symptomless rind pieces (approx. 0.75 cm in diameter, 10 pieces/fruit, 10 fruits each treatment) were surface-sterilized and plated on water agar.

RESULTS

Presence of Perithecia

As shown in Table 1, perithecia of the fungus were widely present in various locations and in different varieties of citrus.

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Table 1. Presence of perithecia of *Guignardia citricarpa* on old dead leaves collected from orchard floor of various locations

Area in the island	Location of orchard	Variety of citrus
North	San-Hsia, Taipei	Ponkan, Tankan
	Hsin-Tien, Taipei	Tankan
	Hsin-Pu, Hsin-chu	Ponkan
	Hsin-Pu, Hsin-chu	Navel orange
Middle	Shen-Kang, Taichung	Ponkan
	Tung-Shu, Taichung	Ponkan
	Ta-Tsun, Chan-Hua	Ponkan, Valencia
South	Mei-San, Chia-I	Valencia
	Chia-I, Chia-I	Matou-wen-tan

Effect of Covering Fruits of Ponkan for Various Intervals after Flowering on the Number of Black Spot Lesions

Table 2 shows the date and results of covering fruits. Highly significant differences existed between fruits covered on or before June 12 and fruits covered on or after June 25. While no significant difference existed either between fruits covered on or before June 12 or between fruits covered on or after June 25. *G. citricarpa* was isolated from 6 of 70 lesion-free rind pieces from fruits coverd on April 5, 41 of 100 from June 12 fruits and 83 of 100 from July 26 fruits (Table 6). Thus, bagging reduced the number of latent infections.

Table 2. Effect of bagging fruits of Ponkan for various intervals after flowering on the number of black spot lesion

Date covered	First lesion counting[a]		Second lesion counting[b]		Significant difference (*t* test)
	No. of fruit in sample	Aver. lesion/fruit	No. of fruit in sample	Aver. lesion/fruit	
1965					
April 5	15	0.00	15	2.20	**June 25, July 26 Aug. 26
17	20	0.55	18	4.00[c]	**Aug. 26 *June 25, July 26
May 1	28	0.68	25	1.96	
15	11	3.45	10	8.00[d]	
29	20	1.10	20	1.65	
June 12	54	1.46	49	2.82	**June 25, July 26 Aug. 26
25	62	6.02	58	10.21	
July 26	81	6.33	78	21.60	
Aug. 26	73	8.07	71	30.45	

a. Lesion counting at the time of harvest.
b. Lesion counting after 14 days incubation.
c. Three fruits totaled 57 lesions.
d. Two fruits totaled 74 lesions.

Spray Experiments for Time of Fruit Infection

Table 3 and 4 show treatments of an experiment in which staggered sprays of Difolatan W-80 were applied to 8 years old orange trees of var. Ponkan. During October and November, fruits were picked from each tree, incubated and

Table 3. Spray date for application of Difolatan W-80 at 2 gr/liter to Ponkan trees

Treatment	1965										
	April		May		June		July		August		
	10	24	10	24	7	23	3	17	1	17	30
A	×	×	×	×	×	×	×	×	×	×	×
B		×	×	×	×	×	×	×	×	×	×
C			×	×	×	×	×	×	×	×	×
D				×	×	×	×	×	×	×	×
E					×	×	×	×	×	×	×
F						×	×	×	×	×	×
G	×	×	×	×	×	×	×	×	×	×	
H	×	×	×	×	×	×	×	×	×		
I	×	×	×	×	×	×	×	×			
J	×	×	×	×	×	×	×				
K	×	×	×	×	×	×					
L	Control-No sprays										

Table 4. Black spot lesions on orange fruits from trees sprayed at various times with Difolatan (see Table 3); randomized block design with 4 replicates of two trees blocks

Treatment	First lesion counting[a]		Second lesion counting[b]		Significant difference
	No. of fruit in sample	Average lesion/fruit	No. of fruit in sample	Average lesion/fruit	
A	384	0.49	376	0.43	
B	183	0.76	169	1.99	
C	840	1.19	818	2.05	
D	435	1.15	416	2.27	
E	324	2.17	317	4.96	
F	740	5.08	677	10.21	**D *E, K
G	622	0.37	614	1.11	
H	473	1.12	415	2.10	
I	306	0.92	299	3.38	
J	547	0.47	499	1.25	
K	347	1.97	307	5.79	*J
L	503	6.13	435	15.32	**K, E *F

a. The lesion counting at the time of harvest.
b. The lesion counting after 14 days incubation.

lesions counted. Since early ripe fruits of Ponkan are easily falling from trees, fruits were harvested beforehand. Four harvests were made throughout the period. Heavier infection was observed among fruits of early ripe. (Table 5.).

Table 5. Black spot lesions on Ponkan fruit harvested at different time

Time of harvest	No. of fruit in sample	Average lesion/fruit	Signficant difference
Oct. 30, 1965	1,515	9.34	**Nov. 17, 23 *Nov. 9
Nov. 9	907	4.74	
17	940	2.73	
23	1,837	2.06	

Sprays applied before June 7 or after July 17 had no significant effect on disease control. Since Difolatan connot be expected to afford protection for more than 4 weeks, it is probable that no infection occurred after August. Drastically less colonies of *G. citricarpa* were isolated from symptomless rind pieces of A fruit than those of F or L fruits. It may be concluded that Difolatan reduced the number of latent infections. (Table 6, b)

Table 6. The appearance of *G. citricarpa* on lesion free rind pieces from various treatments

Treatment		No. of healthy rind piece	Degree of appearance			Percent infected
			Few	Medium	Abundant	
Bagging trial	April 5	64	6	—	—	9.44
	17	73	25	2	—	27
	May 1	61	35	4	—	39
	15	47	3	—	—	6.38
	29	59	31	7	3	41
	June 12	67	33	—	—	33
	25	61	35	3	1	39
	July 26	17	68	6	9	83
	Aug. 26	37	60	3	—	63
Spray trial	A	86	11	3	—	14
	B	80	18	2	—	20
	C	77	23	—	—	23
	D	62	30	6	2	38
	E	77	18	5	—	33
	F	47	48	5	—	53
	G	80	18	2	—	20
	H	59	29	10	2	41
	I	62	31	5	2	38
	J	67	25	6	2	33
	K	33	61	4	2	67
	L	16	60	19	5	84

DISCUSSION AND CONCLUSION

Perithecia of *Guignardia citricarpa* which are considered to be an important source of inoculum have been observed on old dead leaves in varieties of citrus throughout different locations. The evidence seemed to have clarified the problem of the source of inoculum of black spot causal fungus in this island.

The experimental results reveal that both bagging and spraying reduced the number of infections. However, since no information on discharge of spores of ascigerous stage was available, the initiation date of infection could not be certain. Yet, the effective infection seemed to occur in the early June and last for two months. The results from incubation of lesion free rind pieces on water-agar plate for further checking of actual infection confirmed the conclusion from the field experiments.

Severe disease expressing was obtaind when fruit were incubated for 14 days (Table 7). To make the experimental results more revealing, the 14 days incubation is considered to be worthwhile.

Table 7. Number of lesions at the time of harvest and after 14 days incubation

Lesion Counting	Average lesion/fruit
At the time of harvest	1.82
After 14 days incubation	4.23*

* Difference significant at 5% level.

SUMMARY

The presence of mature perithecia of *Guignardia citricarpa* were observed on old dead leaves of different citrus varieties in various localities of the island.

The results of field experiments including staggered spray of Difolatan W-80 and bagging fruits for various intervals after flowering reveals the fact that effective infection occurs in the early June and last for two months.

LITERATURE CITED

(1) McOnie, K. C. 1964. Source of inoculum of *Guignardia citricarpa*, the citrus balck spot pathogen. Phytopathology 54:64-67.

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柑桔黑星病之研究

1. 病原菌侵入期之檢討

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摘　要

由於柑桔黑星病之有性世代（子囊殼）發現於不同地區不同柑桔品種之果園行將腐化之落葉上，證明了該病原菌之有性世代存在於臺灣，並使年來未能解決的該病主要接種源問題得以澄清。

不同時間及次數之大富[redacted]藥劑撒佈及紙袋套果試驗之結果，指出病原菌多數在六月初開始二個月之期間內侵入果實。